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TITLE: Innate Anti-Breast Cancer Activity of (Gamma) / (Delta) T-Cells: A Novel Biological and Clinical Approach to the Treatment of Relapsed or Refractory Breast Cancer

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14. ABSTRACT
We initially identified and characterized a CD2-mediated, interleukin (IL)-12–dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human -T cells. We have since exploited this pathway to develop the methodologies allowing the large-scale ex vivo expansion of viable apoptosis-resistant -T cells – an undertaking until now, not possible. Importantly, we have shown that apoptosis-resistant human -T cells retain significant innate, major histocompatibility complex (MHC)-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including human breast cancer cell lines. Our efforts related to this proposal have remained focused upon testing the hypothesis that -T cells – by virtue of their innate ability to recognize and kill epithelial-derived malignancies – play an important role in regulating the initial growth or spread of breast cancer in vivo. In this progress report, we discuss the findings we have made in the first and second years of this award. The human pre-clinical work is reported here as we have made some important progress in optimizing our ability to expand -T cells from patients with breast cancer. Data derived from animal studies using the syngeneic model of breast cancer are very preliminary but will be discussed briefly in this year's report. Problems encountered in the first two year – and their solutions – are discussed in this annual report.

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Breast cancer; T cells; immunotherapy

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Table of Contents

INTRODUCTION

We initially identified and characterized a CD2-mediated, interleukin (IL)-12–dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human γδ-T cells. We have since exploited this pathway to develop the methodologies allowing the large-scale ex vivo expansion of viable *apoptosis-resistant* γδ-T cells – an undertaking until now, not possible. Importantly, we have shown that apoptosis-resistant human γδ-T cells retain significant innate, major histocompatibility complex (MHC)-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including human breast cancer cell lines. Our efforts related to this proposal have remained focused upon testing the hypothesis that γδ-T cells – by virtue of their innate ability to recognize and kill epithelial-derived malignancies – play an important role in regulating the initial growth or spread of breast cancer in vivo.

BODY

In this period of our grant for which this report is generated (2 February, 2007 to 2 February, 2008) our accomplishments are presented in relation to the following tasks as outlined in the approved Statement of Work.

Task 1: Clinicopathologic correlations. To determine the extent to which γδ-T cell numbers; γδ-T cell innate antitumor capacity and γδ-T cell expansion potential vary as a function of breast cancer clinical stage, clinical progression and clinical response to standard therapy.

FINDINGS:

1. γδ-T cells can be expanded from peripheral blood obtained from patients with metastatic breast cancer who are actively undergoing therapy.

Purpose and Approach: Early data (***Table I***) demonstrate the feasibility of expanding γδ-T cells from *patients with metastatic breast cancer who are actively undergoing therapy*. For these studies, patients were selected for inclusion on the basis that such individuals will be those recruited in future clinical trials. For this study, γδ-T cells were expanded in cultures using the actual clinical grade reagents which we intend to use to generate γδ-T cells in future clinical trials. The "fold expansion" of γδ-T cells after 18 days was calculated as described in detail on *p. 432* of the original application.

Findings and Significance: Data in ***Table I*** indicate that γδ-T cells can be expanded from patients with metastatic breast cancer who are actively undergoing therapy. Importantly, the magnitude of γδ-T cell expansion in this feasibility study (ranging from near 50 to over 100 fold) is approximately within the range of our estimated (minimum) required cellular expansions in future clinical trials. Thus, while these studies demonstrate the feasibility of expanding γδ-T cells from patients with metastatic breast cancer (who are actively undergoing therapy), these findings also serve to emphasize the importance of optimizing the expansion of γδ-T cells derived from patients with breast cancer. We are now accruing larger numbers of donors in ongoing studies – including donors with earlier stage disease and healthy controls.

Table I Expansion of γδ-T cells from peripheral blood obtained from patients with metastatic breast cancer receiving chemotherapy and/or hormonal therapy.

Patient code	Age	Active Therapy	γδ-T cell Fold Expansion
WEJA	42	yes	***56***
FRLI	62	yes	***119***
RHDO	50	yes	***45***

2. Ex vivo expanded γδ-T cells derived from patients with metastatic breast cancer (actively undergoing therapy) retain cytolytic activity against human breast cancer cells *in vitro*.

Purpose and Approach: Data shown in ***Figure 1*** are provided to demonstrate that *ex vivo* expanded γδ-T cells derived from patients can kill human breast cancer cells *in vitro*. For these studies, γδ-T cells were isolated from the cultures derived from two patients studied above. After expansion, γδ-T cells were purified over an immunomagnetic column. Cytotoxicity assays were performed as previously described using human breast cancer cell line T-47D as target cells for patient-derived γδ-T cells. Data are presented as the percent specific lysis of T-47D target cells upon addition of effector γδ-T cells at the indicated E:T ratios (1:1 to 10:1).

Findings and Significance: Data from ***Figure 1*** indicate that it is indeed possible to expand breast cancer-reactive γδ-T cells from patients with metastatic breast cancer. Thus, in conjunction with the expansion studies presented above, these findings suggest that future clinical trials are indeed feasible.

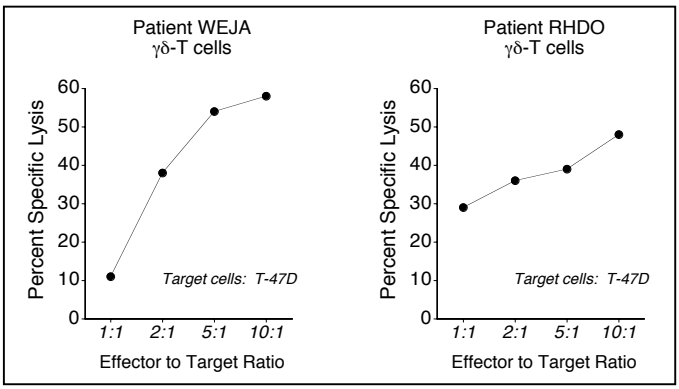

***Figure 1.* γδ-T cells from two separate patients with metastatic breast cancer display cytotoxic activity against human breast cancer cell line T-47D.** γδ-T cells were first expanded (described above) then purified from peripheral blood mononuclear cell cultures derived from patient WEJA (*left*) and patient RHDO (*right*). Purified γδ-T cells were then assessed for their *in vitro* antitumor activity measured against human breast cancer cell line T-47D as previously described. Data are presented as percent specific lysis of T-47D target cells after 4 hr co-culture with the corresponding effector γδ-T cells. Effector to target ratios ranged from 1:1 to 10:1.

Task 2: Basic tumor immunobiology. To further refine our understanding of the in vitro biology (recognition and effector functions) of the antitumor cytotoxicity mediated by human γδ-T cells against human breast cancer cells.

Studies have been performed to confirm that γδ-T cells expanded from patients with breast cancer do indeed kill tumor cells in a T-cell receptor-dependant manner, similar to γδ-T cells obtained from healthy donors. Similarly, we have also confirmed that killing of tumor cells by γδ-T cells derived from patients occurs through the perforin/granzyme pathway, similar to γδ-T cells obtained from healthy donors. We are in the process of analyzing data examining how key activation markers, adhesion molecules as well as cytokine production differ between γδ-T cells obtained from healthy donors and those obtained from patients with breast cancer. These studies are ongoing.

Task 3: Pre-clinical models for the adoptive cellular immunotherapy of breast cancer. To determine the extent to which γδ-T cells can regulate the growth and metastasis of breast cancer cells in vivo using pre-clinical animal models.

Treatment of tumor-bearing animals with human γδ-T cells (xenograft model). Studies in this sub-task are now underway and will be reported with the next annual report.

Mouse syngeneic breast cancer model. Tumorigenic mouse breast cancer cells line 4T1 (derived from BALB/c) have been used to establish disease in syngeneic BALB/c animals. Using two approaches (proof-of-concept studies and adoptive immunotherapy studies), we are now examining the extent to which murine gamma/delta T-cells can prevent the growth or metastasis of 4T1 cells or other tumor cells, *in vivo*.

Proof-of-concept studies. As initially proposed, we predicted that mice depleted of γδ-T cells using the GL3 anti-γδ T cell receptor (TCR) antibody would have more rapid disease progression when challenged with tumorigenic cancer cells. Our studies now show that tumor cells implanted in mice depleted of γδ-T cells using the GL3 anti-γδ TCR antibody do indeed appear to grow faster. These studies are now being confirmed in larger numbers of mice to achieve statistical significance. We are also comparing the routes by which tumor cells are introduced (subcutaneous-skin; mammary fat pad; intravenous-disseminated disease). Our initial studies were performed using a commercially available anti-γδ TCR antibody. We have noted that the cost of the commercially available reagent has proven to be prohibitive – especially in studies where larger numbers of mice are required. Accordingly, we have now acquired the hybridoma which makes the GL3 antibody and have produced the purified antibody for use in the larger studies now underway.

Studies to assess immunotherapeutic potential of adoptively transferred γδ-T cells in the setting of established disease. Here we are determining the extent to which adoptively transferred γδ-T cells can moderate growth or metastasis of established tumor cells. We have now established a colony of BALB/c mice which are deficient in αβ-T cells (founder animals purchased from Jackson Labs). Accordingly, all T cells from these mice are γδ-T cells, making the separation of γδ-T cells from αβ-T cells unnecessary. This is a commonly used approach that assures that the T cells expanded from the spleen cell preparation are all γδ-T cells and not contaminated with αβ-T cells. This is key, since the original studies performed using γδ-T cells isolated from wild type mice were difficult to interpret given that the number of γδ-T cells readily obtainable from such mice were very low. This approach to generating γδ-T cells for cellular therapy then assures us that we will have a pure population of γδ-T cells for adoptive transfer into tumor-bearing mice. We are also in the process of breeding these αβ-T cell deficient BALB/c mice with BALB/c mice which express green fluorescence protein (GFP) yielding BALB/c mice with GFP+ γδ-T cells. These mice will be used to generate γδ-T cells to be used subsequently in tracking studies when introduced into tumor-bearing mice.

KEY RESEARCH ACCOMPLISHMENTS

Data in ***Table I*** indicate that γδ-T cells can be expanded from patients with metastatic breast cancer who are actively undergoing therapy. Data from ***Figure 1*** indicate that it is indeed possible to expand breast cancer-reactive γδ-T cells from patients with metastatic breast cancer. Thus, in conjunction with the expansion studies, these findings suggest that future clinical trials are indeed feasible.

Data support our prediction that tumorigenic 4T1 breast cancer cells grow more rapidly in mice depleted of γδ-T cells where our studies show that 4T1 cells implanted in mice depleted of γδ-T cells using the GL3 anti-γδ TCR antibody do indeed appear to grow faster. This is an important proof of concept finding which strongly supports our underlying hypothesis.

Immunotherapy studies in the syngeneic mouse model at this point are inconclusive as we have not been able to reliably generate the larger numbers of γδ-T cells needed for the larger studies. We have now established a colony of BALB/c mice which are deficient in αβ-T cells, allowing us to generate large numbers of highly purified γδ-T cells from such animals.

REPORTABLE OUTCOMES none to date

CONCLUSION none to date

REFERENCES none

APPENDIX none